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Research Article

Development and Validation of a Simultaneous HPLC Method for Quantification of Amlodipine Besylate and Metoprolol Tartrate in Tablets

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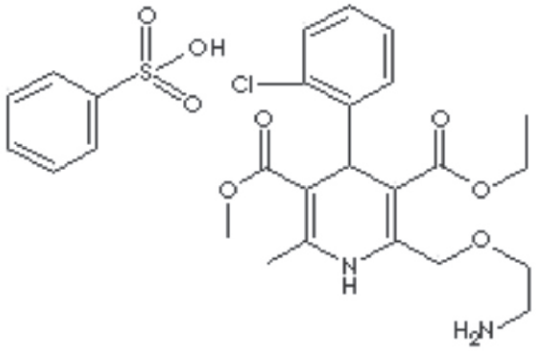
Abstract

A reverse phase method has been developed for the quantitative estimation of amlodipine besylate and metoprolol tartrate in tablet. The quantification was carried out using RP stainless steel column ODS C18 250 x 4.6 x 5 μ L1 packing in isocratic mode with mobile phase containing 0.03 M phosphate buffer and acetonitrile in the ratio of 32: 68 (pH 3.5). Flow rate of 1.2 ml/min and the detection wavelength were set at 230 nm and the linearity was found to be in the range of 8-12 µg/ml for amlodipine besylate and metoprolol tartrate. The proposed method was found to be simple, precise, accurate, and reproducible for the estimation of amlodipine besylate and metoprolol tartrate.

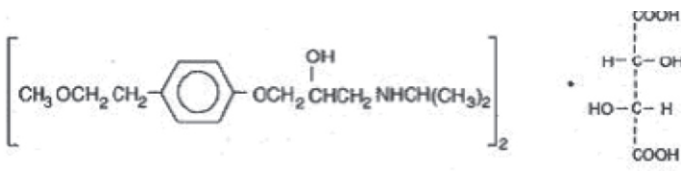
Keywords: Amlodipine besylate, metoprolol tartrate, method development, validation, high performance liquid chromatography

Introduction

Amlodipine besylate is called a calcium channel blocker. These drugs work by blocking the calcium needed for muscle contraction in either primarily heart muscle or primarily arterial muscle. Its overall effect is to relax the arterial muscles so that they dilate and the blood pressure within them drops. Hypotension (blood pressure dropping too low) generally does not occur with amlodipine besylate unless it is combined with another drug that drops blood pressure.



Other drugs of this type might include: beta-blockers such as metoprolol tartrate. Metoprolol tartrate USP is (±)-1-(Isopropyl amino)-3-[p-(2-methoxyethyl) phenoxy]-2-propanol L-(+)-tartrate (2:1) salt.



Materials and Methods

Materials

Amlodipine besylate was obtained from Kopran Limited, Mahad and Metoprolol tartrate from CIPLA Limited, Kurkumb, India. HPLC grade acetonitrile, AR grade formic Acid was procured from Merck, India and Bransted HPLC water was used as received.

HPLC analysis

The analysis was performed on a chromatographic system of Agilent 1100 series G1314B-UV Detector, G1310 A isocratic pump equipped with auto sampler and Ezchrome software version 3.2.1. The chromatographic column was RP stainless steel ODS C18 250 × 4.6 × 5μ L1 packing. HPLC instrument was operated at ambient temperature. The flow rate of the mobile phase was maintained at 1.2 ml/min. Detection was carried out at 230 nm and the injection volume was 20 µl. Retention time of amlodipine besylate was about 3.99 min and that of metoprolol tartrate was 3.07 min. Run time was set for 10 min.

Amlodipine besylate standard solution

Accurately weighed 25 mg of amlodipine besylate tablet was taken into a 25 ml volumetric flask. 5 ml of mobile phase was added to it, sonicated to dissolve and diluted to volume with mobile phase and mixed thoroughly (Solution A).

Amlodipine besylate standard stock solution

One milliliter of Solution A was diluted to 100 ml with the mobile phase.

Metoprolol tartrate standard solution

Metoprolol tablet (25 mg) was taken into a 25 ml volumetric flask.

5 ml of mobile phase was transferred and, shaken thoroughly, sonicated to dissolve, and diluted to volume with mobile phase and mixed thoroughly (Solution B).

Metoprolol tartrate standard stock solution

One milliliter of solution B was diluted in to 100 ml volumetric flask and made up the volume with mobile phase.

Mix standard solution

One milliliter of each Solution A and Solution B was diluted to 100 ml with the mobile phase.

Results and Discussion

Linearity

The developed method has been validated as per ICH guidelines Each 20 ml of the standard solution of amlodipine besylate and metoprolol tartrate in the concentration range of 8-12 mg/ml each were injected into the chromatographic system. The chromatograms were developed and the peak area was determined for each concentration of the drug solution. Calibration curves of amlodipine besylate and metoprolol tartrate were obtained by plotting the peak area ratio versus the applied concentrations of amlodipine besylate (Fig. 1) and metoprolol tartrate (Fig. 2).

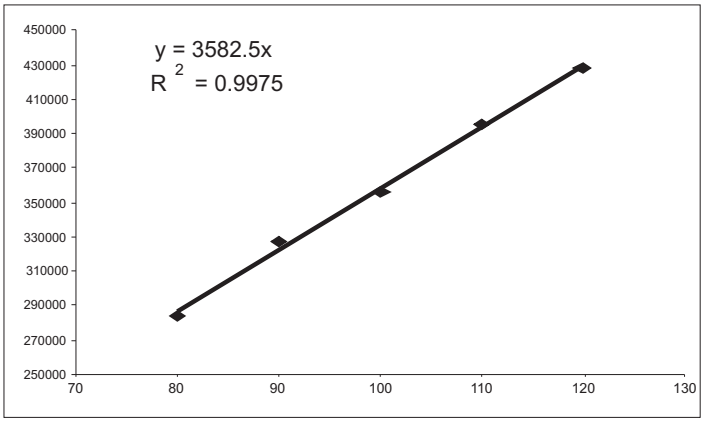


Fig. 1: Linearity graph of Amlodipine Besylate

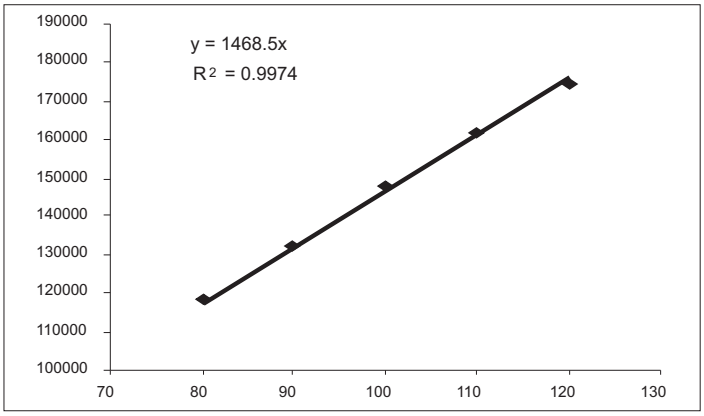


Fig. 2: Linearity of Metoprolol tartrate

The resultant correlation coefficient (R^2) should be $\geq$0.99 for amlodipine besylate and metoprolol tartrate. As per linearity graph of amlodipine besylate and metoprolol tartrate, they were linear with co-efficient of correlation (R^2) of more than 0.99. The linearity within the range of (80 % to 120%), the standard limits concentration is established.

Precision

Repeatability of the method was checked by injecting six replicate injections of the solution 20µL each of amlodipine besylate and metoprolol tartrate, respectively and the RSD was found to be 0.41 and 0.69%. The relative standard deviation of reproducibility and repeatability with respect to peak area and retention time are well within the acceptance criteria. The resolution between amlodipine besylate (Table 1) and metoprolol tartrate (Table 2) was found 5.0 which was more than 1.5 and hence, the method was suitable.

Table 1: Resolution of the two drugs under study

S.N	Amlodipine besylate			Metoprolol tartrate			
	RT	T.P	T.F	RT	T.P	T.F	Resolution
1	3.99	5527	1.56	3.07	6657	1.41	5.01
2	3.99	5643	1.54	3.07	6746	1.45	5.08
3	3.99	5612	1.54	3.07	6595	1.46	5.05
4	3.99	5631	1.52	3.07	6659	1.43	5.06
5	3.99	5515	1.57	3.07	6675	1.41	5.01
6	3.99	5495	1.54	3.07	6588	1.46	5.01

RT= Retention time; TP = Theoretical plates; TF= Tailing Factor.

Accuracy

The accuracy of the method was tested by carrying out recovery studies at different spiked levels. The estimation was carried out as described earlier. At each level, three determinations were performed and results obtained. The amounts recovered and the values of percent recovery were calculated. The results for amlodipine besylate and metoprolol tartrate have been displayed in Table 3 and Table 4.

Table 3: Accuracy data of amlodopine besylate

Conc. Level	Area of un spiked sample	Area of spiked sample	Corrected area	Mean	%RSD	%Accuracy	%Recovery
	345150	597929	252779				
80%	345150	600821	255671	255491	1.03	74.02	92.53
	345150	603175	258025				
	345150	681425	336275				
100%	345150	682421	337271	336510	0.20	97.50	97.50
	345150	681135	335985				
	345150	763524	418374				
120%	345150	768814	423664	423156	1.08	122.60	102.17
	345150	772581	427431				

Table 4: Metoprolol tartrate accuracy data of the method

Conc. Level	Area of un spiked sample	Area of spiked sample	Corrected area	Mean	%RSD	%Accuracy	%Recovery
	152131	271209	119078				
80%	152131	275162	123031	122048	2.14	80.23	100.28
	152131	276166	124035				
	152131	303068	150937				
100%	152131	303124	150993	151195	0.26	99.38	99.38
	152131	303786	151655				
	152131	332950	180819				
120%	152131	333777	181646	181841	0.62	119.53	99.61
	152131	335189	183058				

The accuracy and recovery results obtained with all the three different concentration levels applied (80%,100% & 120%) are well within the acceptance criteria which shows that the method is accurate.

Specificity

The specificity of the method was checked for the interference of retention time of a blank solution (without any sample) and then a drug solution of 20 µL was injected into the column, under optimized chromatographic conditions, to demonstrate the separation of both amlodipine besylate and metoprolol tartrate. There was no interference of blank on retention time of amlodipine besylate and metoprolol tartrate.

Resolution

The resolution (Table 5 and Table 6) between the peaks of amlodipine besylate and metoprolol tartrate should not be less than 1.5. The method was found to be specific and also confirmed (Fig. 3).

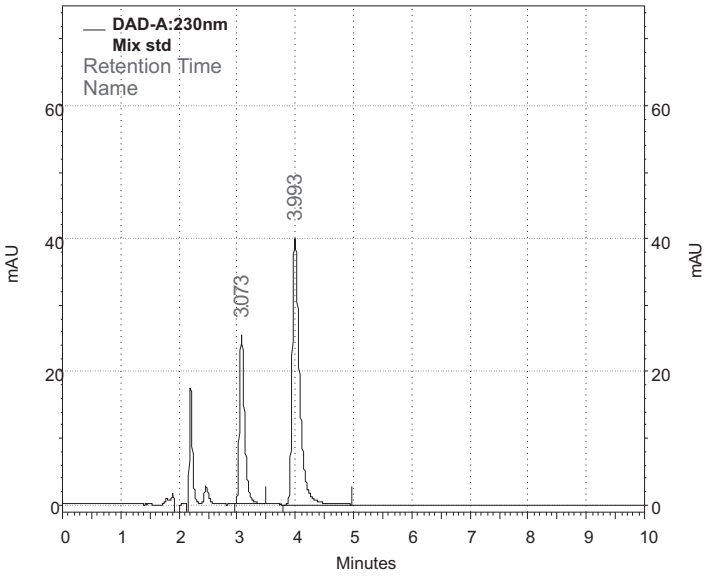


Fig.3: Typical chromatogram of the mixture of amlodipine besylate and metaprolol

Table 5: Resolution of the two drugs by Analyst-I

S.N	Amlodipine besylate			Metoprolol tartrate			
	RT	T.P	T.F	RT	T.P	T.F	Resolution
1	3.99	5527	1.56	3.07	6657	1.41	5.01
2	3.99	5643	1.54	3.07	6746	1.45	5.08
3	3.99	5612	1.54	3.07	6595	1.46	5.05
4	3.99	5631	1.52	3.07	6659	1.43	5.06
5	3.99	5515	1.57	3.07	6675	1.41	5.01
6	3.99	5495	1.54	3.07	6588	1.46	5.01

Table 6: Resolution of the two drugs by Analyst-II

S.N	Amlodipine besylate			Metoprolol tartrate			
	RT	T.P	T.F	RT	T.P	T.F	Resolution
1	3.99	5841	1.55	3.07	6761	1.48	5.10
2	3.99	5472	1.53	3.07	6618	1.44	5.01
3	3.99	5450	1.52	3.07	6594	1.42	5.00
4	3.99	5495	1.54	3.07	6588	1.46	5.01
5	3.99	5449	1.52	3.07	6582	1.43	5.00
6	4.00	5973	1.57	3.08	7140	1.45	5.22

Limit of Quantization (LOQ)

It is the smallest level of analyte that gives a measurable response and standard deviation and slope for the peak area responses was calculated (Table 7 and Table 8).

Table 7: % RSD of each concentration level of amlodipine besylate.

Inj. No.	% RSD of each concentration level					
	Conc. ppm	Sample ID	Peak area response	Mean	%RSD NMT 2.0	Standard Deviation
1.	1.0 ppm	Rep1	33287	34432	2.81	S.D = 32334
		Rep2	32543			
		Rep3	31468			
2.	2.0 ppm	Rep1	64688	64980	0.48	
		Rep2	65310			
		Rep3	64942			
3.	3.0 ppm	Rep1	98441	97100	1.01	Slope= 32334
		Rep2	95537			
		Rep3	96767			
		Rep4	96849			
		Rep5	97301			
		Rep6	97707			

Table 8: % RSD of each concentration level of Metoprolol tartrate.

Inj. No.	%RSD of each concentration level					
	Conc. ppm	Sample ID	Peak area response	Mean	%RSD NMT 2.0	Standard Deviation
1.	1.0 ppm	Rep1	14065	13805	1.88	S.D= 13639
		Rep2	13546			
		Rep3	13804			
2.	2.0 ppm	Rep1	27547	27750	0.65	
		Rep2	27891			
		Rep3	27813			
3.	3.0 ppm	Rep1	41487	41082	0.92	Slope =13638
		Rep2	40756			
		Rep3	41178			
		Rep4	41356			
		Rep5	41217			
		Rep6	40501			

Solution Stability

The changes in area response of the test material will be monitored throughout the validation. For solution stability (Table 9); each solution was injected after 8 h and 40 h.

Table 9: The solution stability data

Time (hrs)	Amlodipine besylate			Metoprolol tartrate			
	RT	T.P	T.F	RT	T.P	T.F	Resolution
8	3.99	5841	1.55	3.07	6761	1.48	5.10
16	3.99	5472	1.53	3.07	6618	1.44	5.01
24	3.99	5450	1.52	3.07	6594	1.42	5.00
32	3.99	5495	1.54	3.07	6588	1.46	5.01
40	3.99	5449	1.52	3.07	6582	1.43	5.00

RT= Retention time; TP = Theoretical plates; TF= Tailing Factor

Conclusion

The developed method was validated in terms of accuracy, linearity and precision. A good linear relationship was observed for amlodipine besylate and metoprolol tartrate in the concentration ranges of 8-12 µg/ml. The correlation coefficient for amlodipine besylate and metoprolol tartrate was found to be 0.99. Selectivity experiment showed that there is no interference or overlapping of the peaks either due to diluents with the main peak of amlodipine besylate and metoprolol tartrate.

The percentage RSD for precision is <2 which confirms that method is sufficiently precise and the total runtime required for the method is only 10 min for eluting amlodipine besylate and metoprolol tartrate. The proposed method is simple, fast, accurate, and precise and can be used for routine analysis in quality control for amlodipine besylate and metoprolol tartrate.

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